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Changes in the amino acid profiles during embryonic development of the blacklip abalone (*Haliotis rubra*)

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Abstract − Changes in the total amino acid (TAA) and the free amino acid (FAA) contents during embryonic development, through newly spawned eggs, to pre-settled larvae of blacklip abalone (*Haliotis rubra*) are described. The TAA (protein bound + free) and the FAA contents increased prior to hatching but decreased towards settlement, but the changes were not always significant between different stages of development. Threonine, arginine, lysine and leucine accounted for nearly 50 % of the total essential amino acids (TEAA) in all developmental stages. The mean FAA content of newly spawned eggs was 262.8 ± 28.2 pmol·ind^{-1} and accounted for 11.5 ± 8.3 % of the TAA. Free essential amino acid (FEAA) content increased significantly as development progressed ($P < 0.05$), in which threonine, arginine and lysine accounted for over 63 % of this pool. In all developmental stages, the FAA pool was dominated by the non-essential amino acids taurine + proline which accounted for 79.5 % of the total. Generally, the FAA accounted for between 10 to 15 % of the TAA in the different developmental stages of blacklip abalone. All evidence appears to indicate that in blacklip abalone the energy requirements during early ontogeny are mostly met with from the lipid reserves, and that there is a tendency to conserve amino acids until pre-settlement. © 2001 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS

amino acids / eggs / embryonic development / blacklip abalone / *Haliotis rubra* / larvae

Résumé − Modifications des profils d'acides aminés durant le développement embryonnaire de l'ormeau (*Haliotis rubra*). Les modifications de la composition en acides aminés totaux (TAA) et en acides aminés libres (FAA) sont décrites durant le développement embryonnaire des œufs, entre la ponte et le stade de fixation larvaire des ormeaux (*Haliotis rubra*). Les contenus en TAA (protéines libres et associées) et en FAA augmentent avant l'éclosion mais diminuent vers la fixation larvaire, mais les modifications ne sont pas toujours significatives entre les différents stades de développement. La thréonine, l'arginine, la lysine et la leucine comptent pour près de 50 % des acides aminés essentiels totaux (TEAA) à tous les stades de développement. La quantité moyenne de FAA des œufs nouvellement pondus est de 262,8 ± 28,2 pmol·ind^{-1} et compte pour 11,5 ± 8,3 % des TAA. La quantité d'acides aminés libres essentiels (FEAA) augmente significativement au cours du développement ($p < 0,05$) ; en particulier, la thréonine, l'arginine et la lysine y comptent pour plus de 63 %. Dans tous les stades de développement, le groupe des FAA est dominé par les acides aminés non essentiels taurine + proline qui compte pour 79,5 % du total. Généralement, les FAA forment de 10 à 15 % des TAA chez les différents stades de développement de cet ormeau. Tout semble indiquer que chez cet ormeau, l'énergie nécessaire au développement ontogénique est puisée principalement dans les réserves lipidiques, et qu'il y a une tendance à conserver les acides aminés jusqu'au stade de fixation larvaire. © 2001 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS

acides aminés / œufs / développement embryonnaire / ormeau / *Haliotis rubra* / larves

1. INTRODUCTION

Amino acids are precursors of proteins and also act as an energy source. Previous investigations have shown that deficiencies or excess of one or more essential amino acids limit protein synthesis and growth or both. Prior to this study, most of the investigations on abalone were mainly focused on the

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nutritional requirements, particularly, the effect of diet on growth, the development of nutritionally balanced and effective artificial feeds for juveniles (Knauer et al., 1993; Mai et al., 1994; Fleming et al., 1996; Viana et al., 1996) and seasonal changes in the chemical composition of muscle and viscera (Hwang et al., 1997; Chiou et al., 2001).

The importance of amino acids in the nutritional physiology of the early life stages of marine fish has been well documented. It has been shown that free amino acids (FAA) are abundant in eggs of marine fish which lay pelagic eggs and these primarily provide an energy source during early development (Ronnestad et al., 1999). The opposite trend has been reported for eggs of freshwater fish (Gunasekera et al., 1999). In marine invertebrates on the other hand, FAAs have been known to represent an osmotic adaptation to seawater, but the composition of the FAA pool is known to vary amongst species (Gilles, 1979).

Studies on changes in amino acid profiles in molluscs in relation to early ontogeny are scarce. Records on the biochemical and energy changes during development were reported for marine bivalves, scallop (Yance et al., 1982; Whyte el al., 1990; Farias et al., 1998) and oyster (Ferreiro et al., 1990). Changes in metabolic composition during the reproductive cycle, particularly of the carcass through maturation, were reported for black abalone *Haliotis cracheroidii* (Webber, 1970). The proximate composition and amino acid composition of the adult muscle of various abalone species are also known (Olley and Thrower, 1977; Pierce and Politis, 1990; Hwang et al., 1997; Chiou et al., 2001). The amino acid profile of fertilised eggs and changes thereof with development are known to be important from many aspects. The changes in relation to development are indicative of the use of energy substrates during early ontogeny and can be used in improving broodstock condition, especially in the case of species of aquaculture importance. No systematic investigation has been made of the changes occurring in the amino acid pool during egg and larval development of any abalone species. The present study was instigated to evaluate changes in the amino acid profiles in relation to development from egg to settled larvae of blacklip abalone *H. rubra*. It is expected that the results of the present findings will contribute to the knowledge on broodstock quality as well as larval nutrition in aquaculture, and further understanding of the metabolism during early development in molluscs which is poorly documented.

2. MATERIALS AND METHODS

This study is based on samples of unfertilised and fertilised eggs, hatched larvae and pre-settled larvae of artificially propagated blacklip abalone, obtained during the routine operations of the hatchery of the Southern Ocean Mariculture, Port Fairy, Victoria, Australia. Sample collection was done during the 97/98 spawning season which occurred about December to March. The material used in this study was obtained from eight spawnings, from broodstcok collected from the wild (142°15’ E; 38°21’ S).

2.1. Collection of propagated larvae

Spawning of abalone was triggered by using a combination of the desiccation and the UV irradiation methods (Kikuchi and Uki, 1974). Spawning occurred at least 6 h after treatment. Spawned eggs and sperm were immediately separated from the spawners using a siphon. Fresh eggs were washed with clean seawater (35) and a portion of them was taken for analysis. Artificial fertilisation was effected by mixing a known number, estimated using a sub-sampling technique of eggs, with an appropriate concentration of fresh sperm in seawater. Fertilised eggs were rinsed with clean seawater to remove excess sperm.

Batches of eggs from individual spawners were incubated and the resulting larvae reared separately. Samples of fertilised eggs were collected 20 min after fertilisation, while samples for the hatched stage were obtained 2 h after hatching, which occurred generally about 18 h after fertilisation at 18 °C. Larval rearing was done using a flow-through system for 5 d with pre-settled larvae being determined by the formation of the third cephalic tentacle, in this case an average 5 d post hatch. Overall, approximately, 2 g wet weight sample of each of the developmental stages from each spawning was taken for chemical analyses in this study. New samples were washed with 0.1-µm filtered UV treated seawater and transported to the laboratory in an esky cooler box. A blotting technique was used to remove excess water before samples were frozen at –30 °C until further analysis. A known wet weight of eggs or larvae from a thawed sample was separately taken for the determination of the number of eggs or larvae in the sample. Thawed samples for chemical analyses and for estimating the number were treated in the same manner. Separate thawed egg and/or larval samples from each female were used for determining the moisture and protein, lipid, total amino acid and free amino acid contents.

2.2. Proximate analysis

Moisture, protein and lipid analyses of eggs and larval samples were carried out according to the methods specified by the Association of Official Analytical Chemist (AOAC, 1990). Each determination on a sample was done in duplicate only, due to limitations of the amount of material available.

2.3. Amino acids

The common sample preparation procedures used previously in our laboratory for total and free amino acids (TAA and FAA) analyses were followed (Gunasekera et al., 1998), with a 100-mg wet weight sample (dry) being used for each of TAA and FAA estimations.

Briefly, total amino acid (TAA) was analysed by hydrolysing a sample with 6 N HCl for 24 h at 100 °C under anaerobic conditions. An appropriate amount of the hydrolysate was taken, diluted with 0.5 M borate buffer, pH adjusted to 8.5, and was filtered through a 25-μm membrane filter. Free amino acid was estimated by reacting a sample with 6 % trichloroacetic acid, homogenised (Ika-laboratorik homogeniser) in an ice sealed container at a speed of 24 000 rpm, and centrifuged for 20 min at 8 400 *g*. The resulting supernatant was separated, freeze dried, and reconstituted in 0.25 M borate buffer, pH adjusted to 8.5, and was filtered through a 25-μm membrane filter.

L-Hydroxyproline was used as an internal standard to analyse both total and free amino acids. The pH adjusted samples were reacted with 9-fluoroenylmethyl chloroformate (FMOC) to form FMOC derivates by means of a fully automated GBC LC 1610 Autosampler. Pre-column fluorescence method was applied to analyse FMOC derivates by using an automated GBC LC 1150 HPLC, equipped with a Hypersil column (150 mm L × 4.6 mm ID) (GBC Scientific Equipment, Australia). Resulting peaks were analysed using a Winchrom software package (GBC Scientific Equipment, Australia). The method used did not allow the determination of tryptophan. In FAA analysis, the peaks for taurine and proline were inseparable. As such we have expressed the results as taurine + proline.

2.4. Data handling

Eggs and/or larvae from each spawn were treated as a replicate. The results on amino acid content of eggs and larvae were subjected to one-way ANOVA followed by the Duncan multiple range test. Statistical analyses were performed using the SPSS 8.2 statistical package SPSS (Australasia).

3. RESULTS

The present study was carried out on samples obtained from eight spawns of blacklip abalone. The shell length and the total weight of abalone females used, ranged from 12.0 to 14.1 cm and 296.4 to 445.9 g, respectively.

The changes in mean weight and proximate composition in relation to development of blacklip abalone are given in *table I*. The moisture content increased significantly in pre-settled larvae. Similarly, the protein content of unfertilised eggs increased from 0.06 to 0.43 $\mu g \cdot ind^{-1}$ in pre-settled larvae, even though the decrease of protein content from hatched towards pre-settled larvae was not significant ($P > 0.05$).

The total amino acid (TAA) and the free amino acids (FAA) profiles in relation to the development of blacklip abalone are presented in *tables II* and *III*. There was an increase in total essential amino acids (TEAA) content as development progressed, while the TAA and total non-essential amino acids (TNEAA) content increased prior to hatching and decreased towards settlement. It is noteworthy that the TEAA content of fertilised eggs was significantly higher than in non-fertilised eggs, but the increase in TNEAA content toward hatching was not significant ($P > 0.05$) (*table II*).

Of the TEAA, except lysine which did not change significantly with development, changes were evident in all the other EAA between some of the developmental changes. However, none of the TEAA showed a significant, progressive increase with development. The EAA found in highest quantity in unfertilised eggs, fertilised eggs and pre-settled larvae were threonine, lysine and leucine, and in hatched larvae were threonine, arginine and leucine. In all developmental stages, these amino acids accounted for nearly 50 % of the TEAA.

Of the NEAA, proline was the most dominant in all the developmental stages followed by glutamic acid. Comparable trends were evident amongst TNEAA in relation to development, except that serine, glycine and proline contents differed significantly amongst the different stage of development ($P < 0.05$).

Changes in the FAA pool were also evident during development (*table III*). The free essential amino acids (FEAA), and free non-essential amino acids (FNEAA) were higher in fertilised eggs than in unfertilised ones, but these differences were not significant ($P > 0.05$). The highest content of FAA and FNEAA was found in the newly hatched larvae, while that of FEAA was in pre-settled larvae. There was a significant increase in FAA and FNEAA in hatched larvae followed by a depletion prior to settlement. However, these decreases were not significant ($P > 0.05$). There was no significant difference amongst all individual amino acids in newly spawned eggs and fertilised eggs

Table I. Proximate composition (± SE) of unfertilised and fertilised eggs, hatched and pre-settled larvae of *Haliotis rubra*. Means are based on determination on eight samples from each stage of development (analysed in duplicate; results expressed on a wet-weight basis). Values in each row with the same superscript are not significantly different ($P > 0.05$).

Parameter ($\mu g \cdot ind^{-1}$)	Ufe	Fe	Nhl	Psl
Weight	4.98 ± 0.10^{a}	5.02 ± 0.10^{a}	5.53 ± 0.16^{b}	7.40 ± 0.15^{c}
Moisture	3.77 ± 0.12^{a}	3.72 ± 0.11^{a}	3.72 ± 0.15^{a}	5.04 ± 0.10^{b}
Protein	0.06 ± 0.01^{a}	0.09 ± 0.01^{b}	0.48 ± 0.01^{c}	0.43 ± 0.01^{c}
Lipid	0.81 ± 0.05^{c}	0.73 ± 0.03^{c}	0.61 ± 0.04^{b}	0.48 ± 0.02^{a}

Ufe: Unfertilised egg; Nhl: newly hatched larvae; Fe: fertilised egg; Psl: pre-settled larvae.

Table II. The changes of total amino acids during development expressed as individual egg and larva of *Haliotis rubra* (pmol·ind^{-1}). Values are means ± SE of sixteen replicates. Values in each row with the same superscript are not significantly different ($P > 0.05$) as determined by Duncan's multiple range test. nd: not detected.

Amino Acid	Ufe	Fe	Nhl	Psl
Essential amino acids (EAA)				
Histidine	25.4 ± 3.5^{a}	33.8 ± 3.3^{a}	44.7 ± 5.9^{ab}	63.3 ± 4.7^{b}
Threonine	184.9 ± 15.5^{a}	260.7 ± 15.6^{b}	324.2 ± 17.9^{c}	257.1 ± 13.0^{b}
Arginine	139.2 ± 12.8^{a}	189.1 ± 12.2^{ab}	306.8 ± 20.1^{c}	218.5 ± 10.5^{b}
Valine	99.7 ± 10.3^{a}	144.9 ± 13.1^{b}	168.7 ± 17.9^{bc}	188.3 ± 13.2^{bc}
Methionine	66.6 ± 7.6^{a}	139.2 ± 42.4^{b}	109.1 ± 12.2^{ab}	121.2 ± 9.0^{ab}
Isoleucine	85.5 ± 9.0^{a}	121.3 ± 12.6^{b}	150.0 ± 14.5^{bc}	166.0 ± 12.2^{c}
Leucine	163.5 ± 18.6^{a}	209.9 ± 18.3^{ab}	247.4 ± 31.0^{bc}	310.9 ± 23.3^{c}
Phenylalanine	85.5 ± 11.7^{a}	112.7 ± 13.2^{a}	122.4 ± 15.5^{ab}	155.3 ± 13.1^{ab}
Lysine	173.9 ± 35.6^{a}	259.8 ± 42.1^{a}	211.2 ± 45.4^{a}	315.5 ± 72.9^{a}
Σ EAA	1 024.3 ± 80.3^{a}	1 428.9 ± 82.0^{b}	1 685.1 ± 145.5^{bc}	1 796.1 ± 134.2^{c}
Non-essential amino acids (NEAA)				
Aspartic acid	199.5 ± 17.1^{a}	237.0 ± 19.0^{a}	366.0 ± 27.7^{b}	347.8 ± 27.5^{b}
Glutamic acid	243.3 ± 20.4^{a}	309.2 ± 22.7^{a}	492.5 ± 32.7^{b}	449.4 ± 30.8^{b}
Serine	186.5 ± 14.7^{a}	240.5 ± 13.1^{b}	343.2 ± 17.8^{d}	297.5 ± 15.3^{c}
Glycine	162.5 ± 17.0^{a}	210.1 ± 11.6^{b}	263.5 ± 19.9^{c}	364.7 ± 15.8^{d}
Alanine	178.4 ± 17.0^{a}	229.6 ± 14.3^{ab}	287.3 ± 30.6^{ac}	335.8 ± 22.2^{c}
Taurine + proline	281.8 ± 22.9^{a}	404.2 ± 19.5^{b}	811.5 ± 62.5^{d}	549.0 ± 34.8^{c}
Tyrosine	65.1 ± 10.3^{a}	88.6 ± 10.1^{ab}	94.8 ± 15.7^{ab}	114.5 ± 11.1^{b}
Cystine	nd	nd	nd	468.7 ± 86.0
Σ NEAA	1 287.6 ± 99.7^{a}	1 719.3 ± 92.5^{b}	2 658.9 ± 154.6^{c}	2 458.6 ± 140.2^{c}
Σ TAA	2 311.9 ± 171.3^{a}	3 148.2 ± 161.4^{b}	4 344.4 ± 289.5^{c}	4 254.7 ± 264.7^{c}

Ufe: Unfertilised egg; Nhl: newly hatched larvae; Fe: fertilised egg; Psl: pre-settled larvae.

Table III. The compositions of free amino acid during development expressed as individual egg and larva of *Haliotis rubra* (pmol·ind^{-1}). Values are means ± SE of sixteen replicates. Values in each row with the same superscript are not significantly different ($P > 0.05$) as determined by Duncan's multiple range test. Abbreviations used as for *table II*.

Amino acid	Ufe	Fe	Nhl	Psl
Essential amino acids (EAA)				
Threonine	6.3 ± 0.9^{a}	6.4 ± 0.7^{a}	12.7 ± 1.0^{b}	18.0 ± 1.5^{c}
Arginine	7.2 ± 1.2^{a}	8.8 ± 1.1^{a}	18.6 ± 1.4^{b}	9.3 ± 1.5^{a}
Valine	1.8 ± 0.3^{a}	1.6 ± 0.2^{a}	5.7 ± 0.6^{b}	14.5 ± 2.0^{c}
Methionine	1.2 ± 0.2^{a}	0.9 ± 0.2^{a}	2.4 ± 1.4^{ab}	3.5 ± 1.0^{c}
Isoleucine	2.3 ± 0.3^{a}	2.5 ± 0.3^{a}	7.5 ± 0.8^{b}	9.2 ± 1.5^{b}
Leucine	1.2 ± 0.2^{a}	1.1 ± 0.1^{a}	4.9 ± 0.6^{b}	6.3 ± 1.1^{b}
Phenylalanine	1.6 ± 0.3^{a}	1.5 ± 0.2^{a}	6.9 ± 0.9^{b}	5.3 ± 0.7^{b}
Lysine	4.1 ± 0.8^{a}	5.4 ± 0.6^{a}	15.5 ± 1.6^{b}	24.4 ± 3.2^{c}
Σ FEAA	25.7 ± 3.5^{a}	28.3 ± 2.8^{a}	74.2 ± 6.0^{b}	90.6 ± 8.2^{c}
Non-essential amino acids (NEAA)				
Aspartic acid	2.5 ± 0.4^{a}	2.5 ± 0.3^{a}	3.0 ± 0.3^{ab}	4.1 ± 0.6^{b}
Glutamic acid	3.9 ± 0.6^{a}	4.0 ± 1.9^{a}	7.1 ± 0.5^{a}	13.1 ± 1.9^{b}
Serine	4.5 ± 0.7^{a}	4.7 ± 0.7^{a}	12.1 ± 2.7^{b}	22.7 ± 0.9^{c}
Glycine	9.0 ± 1.9^{a}	5.9 ± 0.9^{a}	7.8 ± 0.7^{a}	9.2 ± 0.7^{a}
Alanine	2.1 ± 0.3^{a}	2.3 ± 0.2^{a}	5.1 ± 0.4^{a}	15.9 ± 3.0^{b}
Taurine + proline	212.1 ± 22.6^{a}	265.5 ± 31.0^{ab}	472.0 ± 33.2^{c}	337.5 ± 30.4^{b}
Tyrosine	3.4 ± 0.6^{a}	2.2 ± 0.3^{a}	9.2 ± 2.3^{b}	15.9 ± 1.1^{c}
Cystine	nd	nd	nd	6.6 ± 0.9
Σ FNEAA	237.1 ± 25.2^{a}	287.2 ± 33.1^{a}	513.3 ± 36.1^{b}	424.2 ± 31.6^{b}
Σ FAA	262.8 ± 28.2^{a}	315.6 ± 35.7^{a}	587.6 ± 40.9^{b}	514.9 ± 35.2^{b}

Table IV. The changes of the ratio of FNEAA, FEAA into free amino acid (FAA) and FAA into total amino acid (TAA) during development per egg and larva of *Haliotis rubra*. Values are means ± SE of sixteen replicates. Values in each row with the same superscript are not significantly different ($P > 0.05$) as determined by Duncan's multiple range test.

Ratio (%)	Ufe	Fe	Nhl	Psl
FNEAA/FAA	90.5 ± 0.6^{c}	90.8 ± 0.5^{c}	87.4 ± 0.5^{b}	82.1 ± 1.5^{a}
FEAA/FAA	9.5 ± 0.6^{a}	9.2 ± 0.4^{a}	12.6 ± 0.5^{b}	17.9 ± 1.5^{c}
FNEAA/TNEAA	18.3 ± 1.9^{a}	18.0 ± 2.6^{a}	30.4 ± 1.4^{a}	15.4 ± 1.0^{a}
FEAA/TEAA	2.7 ± 0.5^{a}	2.1 ± 0.3^{a}	5.0 ± 0.4^{b}	5.7 ± 0.8^{b}
FAA/TAA	11.5 ± 8.3^{a}	10.7 ± 7.6^{a}	14.7 ± 12.6^{a}	11.7 ± 9.2^{a}

($P > 0.05$). On the other hand, only some amino acids remained unchanged in the larval stages.

Threonine, arginine and lysine accounted for over 63 % of the FEAA in unfertilised eggs, fertilised eggs and hatched larvae. Nearly 60 % of the FEAA in pre-settled larvae was accounted for by lysine, threonine and valine. Of the FNEAA, taurine + proline accounted for over 79.5 % in all the developmental stages studied. The other major amino acids were glycine, serine and glutamic acid in eggs, while serine, tyrosine and glycine were in larvae.

Overall, FAA accounted for between 10 to 15 % of the TAA in the different developmental stages of blacklip abalone (*table IV*). However, FEAA represented a much lower proportion of the TAA, but almost doubled in newly hatched (5.0 ± 0.4 %) and newly-settled larvae (5.7 ± 0.8 %) compared to that in unfertilised eggs (2.7 ± 0.5 %) and fertilised eggs (2.1 ± 0.3 %) (*table IV*). Of the FAA pool, NEAA were dominant, and ranged from 82.1 ± 1.5 to 90.8 ± 0.5 % in newly settled larvae and fertilised eggs, respectively. Furthermore, there was a significant decline in the FNEAA/FAA with development and the reverse occurring in FEAA/FAA, when the latter increased from 9.2 ± 0.4 % in fertilised eggs to 17.9 ± 0.5 % in newly-hatched larvae (*table IV*).

4. DISCUSSION

The results of proximate analysis showed that both protein and lipid content changed with development in blacklip abalone. It has been established that protein and lipid are the major energy sources for marine animals in early development and that blacklip abalone is no exception in this respect.

The proximate composition of egg and larvae of blacklip abalone is comparable to that of other abalone species. The net increases in protein and lipid content from day 0 (oocytes) to day 2 (larvae), followed by a decrease toward settlement were reported for red abalone (Jaeckle and Manahan, 1989a). In the present study, the changes in protein content related to changes of TAA, whereas the net increase was noticed from day 0 to hatching, followed by a decrease prior to settlement (*table II*). With this increase in protein content a concurrent increase in TAA content from egg to hatched larvae was also observed. The observation that significant decrease in lipid content occurred with development in blacklip abalone was slightly different to that reported on red abalone (Jaeckle and Manahan, 1989a). In the present study, lipid content decreased gradually as development progressed. As non-feeding larvae, the energy derived from yolk will support development until larvae start to feed. Therefore, a significant decrease of lipid content prior to hatching may indicate its important role as an energy source during cleavage. The concurrent increase in protein content and TAA with ontogeny may be indicative of the fact that in blacklip abalone lipids act as the main energy source during early ontogeny.

As lecithotropic larvae (non-feeding), *Haliotis* larvae cannot feed on particles (Crofts, 1937), but do have the capacity to absorb dissolved organic material (DOM, e.g. amino acids) from seawater (Jaeckle and Manahan, 1989b). These authors also observed that abalone larvae do not use endogenous reserves to meet the total metabolic requirements (Jaeckle and Manahan, 1989a). The ability to absorb amino acids from seawater may contribute to an increase in amino acids content during development. Uptake of amino acid has also been reported for other marine mollusc with lecithotrophic larvae, and can occur 1 h after fertilisation (Manahan, 1983). A previous study on red abalone has shown the cumulative transport of DOM from seawater during development to early juvenile stages could supply an amount of energy equivalent to the initial maternal endowment of energy reserves (Suzuki et al., 1987). Accordingly, for development of *H. rufescens*, the adaptive significance of the large amount of energy initially present in the oocyte may be related to juvenile survivorship, rather than energy metabolism during larval development.

Amongst taxonomic groups, especially in marine invertebrate phyla, variations in the TAA and FAA pools exist. It has been reported for marine invertebrates that the largest variation in amino acid pool occurred always in respect of the non-essential amino acids (Gäde and Grieshaber, 1986). In tissues of blacklip abalone, arginine, leucine, lysine, threonine, valine and isoleucine were the major amino acids, and taurine was not reported (King et al., 1996). However, in the present study taurine + proline were found in significant amounts, in highest quantities, in all the development stages, in the free amino acid pool. Our

observations are in agreement with those on disk abalone, *Haliotis discus* (Watanabe et al., 1992; Watanabe et al., 1993; Hatae et al., 1995), except that during the spawning season when it dropped by about 50 %. It has been suggested that taurine is synthesised by larval stages from precursors to taurine present in micro-algae feeds, and its absence in adult molluscs suggest that this acid is a dietary requirement for adults (Welborn and Manahan, 1995).

Previous studies on other marine invertebrate have shown that some of the non-essential amino acids, such as glycine, alanine, proline, serine, aspartic acid, glutamic acid or taurine are dominant (Crofts, 1937; Webber, 1970). During chemical analysis, asparagine and glutamine are converted to aspartic acid and glutamic acid, respectively. As such relatively high levels of aspartic acid and glutamic acid could be due to these chemical changes during the acid hydrolysis involved in the estimation of the total amino acid contents. The result of our study where non-essential amino acids such as taurine, glycine, serine and glutamic acid are abundant in eggs and larvae of blacklip abalone are comparable to those reported previously for marine invertebrates (Crofts, 1937; Webber, 1970). Taurine and glycine were reported as the main NEAA in the FAA pool in the muscle of New Zealand abalone (Paua) *H. iris* (Bewick et al., 1997). Taurine has also been observed to be the predominant amino acid in the FAA pool of the muscle of *H. tuberculata* and *H. discus* (Mai at al., 1994; Hatae et al., 1995) and in the hemolymph of the bivalves *Pinctada fucata*, *Crassostrea gigas* and *Scapharca subcrenata* (Tjeerdema et al., 1991).

In the present study, there is a significant increase in taurine + proline of the FAA pool after fertilisation, followed by depletion after hatching towards settlement (*table III*). Taurine, the major component of the FAA pool, has been documented in lecithotrophic larvae *H. rufescens* (Jaeckle and Manahan, 1989b) as well as for planktotrophic (feeding) larvae of the bivalve *C. gigas* (Manahan, 1989). The recent finding on molluscan larvae with lecithotrophic development (*H. rufescens*) is that taurine is initially present in the egg in sufficient quantities for larval development to metamorphosis (Welborn and Manahan, 1995). These authors also showed that there is significant synthesis of taurine during development and that feeding increases synthesis as much as 11-fold compared to unfed larvae. For marine invertebrates, the functional roles of taurine are numerous and are still being defined (Huxtable, 1992).

Of the FEAA, a higher level of arginine, threonine and valine in muscle was documented for *H. discus* (Watanabe et al., 1993). In the muscle of *H. iris*, arginine was the second major amino acid in the FAA pool after taurine followed by hydroxylysine, methionine and threonine (Bewick et al., 1997). In muscle and viscera of juvenile *H. diversicolor*, taurine, arginine, glycine, glutamic acid and alanine were found to be dominant in the FAA pool, and an increase in taste active amino acids (arginine, glycine, glutamic acid and alanine) in the autumn and winter months were also recorded (Hwang et al., 1997). Chiou et al. (2001) also found that the same amino acids dominated the FAA pool in muscle and viscera of *H. diversicolor*, and accounted for about 81 to 94 % of the FAA pool in muscle and viscera. In general, the observations on the FAA pool in muscle and viscera of *H. diversicolor* are comparable to the present findings on the different development stages of blacklip abalone.

Certain FAAs have multiple physiological functions independent of osmoregulation and protein synthesis (Mai et al., 1994). Alanine, glycine and arginine, which are normally contained at a higher level in tissues of marine molluscs, are proven to be crucial in energy metabolism by maintaining glycolysis through the formation of opines under hypoxic conditions (Gäde and Grieshaber, 1986). Arginine is also an important endogenous energy compound as a phosphogen (phosphoarginine) in abalone (Tjeerderma et al., 1991). Recent findings have shown that taurine, the most abundant FAA in *Haliotis*, has the same function in anaerobic energy metabolism via the formation of a new end-product of glycolysis, tauropine (Gäde, 1986; Gäde, 1988; Sato et al., 1991; Shilling et al., 1996).

In conclusion, it is evident that blacklip abalone depends primarily on lipid reserves of the egg during early ontogeny. However, during early development the species tends to conserve amino acids, particularly EAA. Accumulation of EAA during development could be augmented by absorption from the external medium, as have been shown for other molluscan species (Manahan, 1983), and this area warrants further investigation. The present study also demonstrated the importance of taurine + proline in the amino acid pool. However, the available evidence in this regard on other molluscan and indeed marine invertebrate, do not appear to be uniform, and further studies are warranted if the role of these amino acids are to be fully understood in early ontogeny of mollusc.

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